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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Wittamer, et al.
Serial No.:	09/905,253
Filed:	July 13, 2001
Entitled:	Natural Ligand of G Protein Coupled Receptor ChemR23 and Uses Thereof

COPY OF PAPERS ORIGINALLY FILED

Attorney Docket No. : 9409/2042
Box: Missing Parts
Commissioner for Patents
Washington, DC 20231

AMENDMENT

Sir:

This is filed in response tot he Notice to File Missing Parts of Nonprovisional Application mailed in the above-noted application on July 30, 2001.

Kindly enter the following amendments and remarks.

In the Specification:

Please amend the specification as follows:

- On a new page, numbered page 64 and immediately following the claims, please insert the following:

-- ABSTRACT

The invention relates to the identification of TIG2, the polypeptide product of **T**azarotene-**I**nduced **G**ene **2**, as a natural ligand of the ChemR23 G protein coupled receptor (GPCR). The invention encompasses the use of the interaction of ChemR23 polypeptides and TIG2 polypeptides as the basis of screening assays for agents that modulate the activity of the ChemR23 receptor. The invention also encompasses diagnostic assays based upon the ChemR23/TIG2 interaction, as well as kits for performing diagnostic and screening assays. --

- On page 18, replace paragraph 8 (lines 21-22) with the following replacement paragraph:

--Figure 8 shows an alignment of the human (SEQ ID NO: 8) and mouse (SEQ ID NO: 10) TIG2 amino acid sequences. Identical amino acids and conservative substitutions are boxed.--

- On page 18, replace paragraph 9 (lines 23-24) with the following replacement paragraph:

-- Figure 9 shows an alignment of human ("tig2"; SEQ ID NO: 31), rat (SEQ ID NO: 32), mouse (SEQ ID NO: 33), sus (SEQ ID NO: 34), bos (SEQ ID NO: 35) and gallus (SEQ ID NO: 36) TIG2 sequences. The figure provides the percent amino acid identity across any two species listed.--

- On page 33, replace paragraph 1 (lines 1-11) with the following replacement paragraph:

-- The substrate for the assay is the peptide Ac-FKKSFKL-NH2 (SEQ ID NO: 37), derived from the myristoylated alanine-rich protein kinase C substrate protein (MARCKS). The K_m of the enzyme for this peptide is approximately 50 μM. Other basic, protein kinase C-selective peptides known in the art can also be used, at a concentration of at least 2 -3 times their K_m. Cofactors required for the assay include calcium, magnesium, ATP, phosphatidylserine and diacylglycerol. Depending upon the intent of the user, the assay can be performed to determine the amount of PKC present (activating conditions) or the amount of active PCK present (non-activating conditions). For most purposes according to the invention, non-activating conditions will be used, such that the PKC that is active in the sample when it is isolated is measured, rather than measuring the PKC that can be activated. For non-activating conditions, calcium is omitted in the assay in favor of EGTA. --

- On page 38, replace the first full paragraph (lines 4-16) with the following replacement paragraph:

-- The NF-κB binding element has the consensus sequence GGGGACTTTCC (SEQ ID NO: 38). A large number of genes have been identified as NF-κB responsive, and their control elements can be linked to a reporter gene to monitor GPCR activity. A small sample of the genes responsive to NF-κB includes those encoding IL-1β (Hiscott et al., 1993, Mol. Cell. Biol. 13: 6231-6240), TNF-α (Shakhov et al., 1990, J. Exp. Med. 171: 35-47), CCR5 (Liu et al., 1998, AIDS Res. Hum. Retroviruses 14: 1509-1519), P-selectin (Pan & McEver, 1995, J. Biol. Chem. 270: 23077-23083), Fas ligand (Matsui et al., 1998, J. Immunol. 161: 3469-3473), GM-CSF (Schreck & Baeuerle, 1990, Mol. Cell. Biol. 10: 1281-1286) and IκBα (Haskill et al., 1991, Cell

65: 1281-1289). Each of these references is incorporated herein by reference. Vectors encoding NF-κB-responsive reporters are also known in the art or can be readily made by one of skill in the art using, for example, synthetic NF-κB elements and a minimal promoter, or using the NF-κB-responsive sequences of a gene known to be subject to NF-κB regulation. Further, NF-κB responsive reporter constructs are commercially available from, for example, CLONTECH. --

REMARKS

The amendments to the specification are made:

1) to introduce SEQ ID Nos. corresponding to nucleotide and amino acid sequences in the accompanying Sequence Listing in compliance with 37 C.F.R. §§1.821-1.825;

2) to correct two obvious typographic errors in the second sentence of paragraph 8 on page 18; and

3) to provide an Abstract in compliance with 37 C.F.R. §1.72(b).

The language of the abstract is drawn directly from the first paragraph of the "summary of the Invention" section on page 4. The amendments add no new matter.

Respectfully submitted,

11/9/01
Date

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Version of amendments marked to show changes:

Page 18, paragraph 8:

Figure 8 shows an alignment of the human (SEQ ID NO: 8) and mouse (SEQ ID NO: 10) TIG2 amino acid sequences. Identical amino acids [are] and [conservatove] conservative substitutions are boxed.

Page 18, paragraph 9:

-Figure 9 shows an alignment of human ("tig2"; SEQ ID NO: 31), rat (SEQ ID NO: 32), mouse (SEQ ID NO: 33), sus (SEQ ID NO: 34), bos (SEQ ID NO: 35) and gallus (SEQ ID NO: 36) TIG2 sequences. The figure provides the percent amino acid identity across any two species listed.

Page 33, paragraph 1:

The substrate for the assay is the peptide Ac-FKKSFKL-NH2 (SEQ ID NO: 37), derived from the myristoylated alanine-rich protein kinase C substrate protein (MARCKS). The K_m of the enzyme for this peptide is approximately 50 μM. Other basic, protein kinase C-selective peptides known in the art can also be used, at a concentration of at least 2 -3 times their K_m. Cofactors required for the assay include calcium, magnesium, ATP, phosphatidylserine and diacylglycerol. Depending upon the intent of the user, the assay can be performed to determine the amount of PKC present (activating conditions) or the amount of active PCK present (non-activating conditions). For most purposes according to the invention, non-activating conditions will be used, such that the PKC that is active in the sample when it is isolated is measured, rather than measuring the PKC that can be activated. For non-activating conditions, calcium is omitted in the assay in favor of EGTA.

Page 38, first full paragraph:

The NF-κB binding element has the consensus sequence GGGGACTTTCC (SEQ ID NO: 38). A large number of genes have been identified as NF-κB responsive, and their control elements can be linked to a reporter gene to monitor GPCR activity. A small sample of the genes responsive to NF-κB includes those encoding IL-1β (Hiscott et al., 1993, Mol. Cell. Biol. 13: 6231-6240), TNF-α (Shakhov et al., 1990, J. Exp. Med. 171: 35-47), CCR5 (Liu et al., 1998, AIDS Res. Hum. Retroviruses 14: 1509-1519), P-selectin (Pan & McEver, 1995, J. Biol. Chem. 270: 23077-23083), Fas ligand (Matsui et al., 1998, J. Immunol. 161: 3469-3473), GM-CSF

(Schreck & Baeuerle, 1990, Mol. Cell. Biol. 10: 1281-1286) and IκBα (Haskill et al., 1991, Cell 65: 1281-1289). Each of these references is incorporated herein by reference. Vectors encoding NF-κB-responsive reporters are also known in the art or can be readily made by one of skill in the art using, for example, synthetic NF-κB elements and a minimal promoter, or using the NF-κB-responsive sequences of a gene known to be subject to NF-κB regulation. Further, NF-κB responsive reporter constructs are commercially available from, for example, CLONTECH.

New page 64:

ABSTRACT

The invention relates to the identification of TIG2, the polypeptide product of **T**azarotene-**I**nduced **G**ene **2**, as a natural ligand of the ChemR23 G protein coupled receptor (GPCR). The invention encompasses the use of the interaction of ChemR23 polypeptides and TIG2 polypeptides as the basis of screening assays for agents that modulate the activity of the ChemR23 receptor. The invention also encompasses diagnostic assays based upon the ChemR23/TIG2 interaction, as well as kits for performing diagnostic and screening assays.